Status of microsatellites as genetic markers in cervids

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Abstract: Microsatellite loci distributing on genome randomly act as effective genetic markers. To date, about 200 microsatellite loci were found in cervids by transferring microsatellite PCR primers derived in bovine, ovine to cervids, as well as a few loci derived directly from deer microsatellite library. These loci have been used in parentage determination, genetic diversity and population structure, population introgression, as genetic marker gestation length and wintering survival *et al.* However, microsatellite loci presently found are untouchable to the demand of application. Future work should include: 1) isolating a large number of cervine microsatellite loci, 2) constructing genetic and physical maps of microsatellite loci. So that microsatelites have a strong base for advanced applications in deer.

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Characteristics and significance of microsatellite DNA

Microsatellite DNA is DNA sequences tandemly repeating by the 2-8bp motifs among eukaryotic genome. Because of their simplicity of repeat motif and patterns, they are also called Short Tandem Repeats (STRs), Simple Sequence Repeats (SSRs) or Simple Sequence Length Polymorphism (SSLPs) (Chin 1996). The Abundance of microsatellite DNA is so large on the eukaryotic genome that a microsatellite locus can be found every 10-50kb (Tautz 1989).

People value the microsatellite loci due to its high polymorphism of fragment length created by its variation of motif repeat number, and meanwhile, to its high conservation of regions flanking the repeat region. PCR primers designed in flanking regions used to amplify the microsatellite locus are also conservative to be transferred to other species in the different genus and even different families (Moore *et al.* 1991). On the other hand, the amplified fragment length is between 100-300bp, which are very applicable to PCR technology. PCR-based analysis of microsatellite DNA extends scales of material, including bloodstain, tissue, sperm stain saliva, skeleton, hair, even urine, feces and highly degraded materials. Doubtless, microsatellite DNA is even valuable for materials collected by non-invasive methods (Flagstad *et al.* 1999; Lee 1996; Lorente *et al.* 1996). Alleles generated by PCR are featured codominant, which are sensitive to study the heterozygote and homozygote. The alleles also pass to later generations according to Mendelian laws. Microsatellit DNA, therefore, becomes a powerful genetic marker and will be widely used.

Methods of obtaining microsatellite loci in deer

There are two ways to obtain microsatellite loci (Hammond *et al.* 1998, DeWoody *et al.* 1995; Engel *et al.* 1996; Menotti-Raymond *et al.* 1999): by screening a microsatellite enriched gene library, and transferring microsatellite loci derived in closely related species. Screening a library is a very complex method. A library containing 300-800bp of target species DNA must be constructed first. Then probes with an interested STR sequence were used to detect and precipitate positive clones in the library. After sequencing the positive clones, PCR primers can be designed in the flanking region. These primer sets are then applied to a population to detect the loci polymorphism, involving the allelic fragment size, genotyping standards, allele number, allelic frequencies, heterozygosity etc (Hammond *et al.* 1998). Some researches also calculate the linkage and construct genetic map and physical map (Menotti-Raymond *et al.* 1999). This method is very effective that hundreds of loci can be obtained at the same time, and the only drawback is expensive. Transferring microsatellite loci from closely related species directly is a cheaper and easier alternative. Most primer sets derived are deposited in large retrieval databases such as GeneBank and other publications as well. We can retrieval primer sets of target species or relative species from this retrieval

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tools and test their effectiveness. It was reported that the evolutionarily closer the species are, the more conservative the microsatellite primers will be (Dallimer 1999). In comparison to the former method, transferring microsatellites has more drawbacks. First of all, microsatellites availability is limited by the research in target or relative species. Secondly, only a proportion of primer sets transferred is effective. That even reduced the availability of primers. Finally, all population statistical data must be investigated after primers transferred to a new species or population.

Presently, most microsatellite primer sets reported effective in cervids are transferred from bovine and ovine (Engel *et al.* 1996; Wilson *et al.* 1999; Roed 1998; Slate *et al.* 1998; Roed *et al.* 1998; Wilson *et al* 1997; Kuhn *et al.* 1996). In some cases, PCR system, including components, amount of template DNA, cycling program needs optimization. The most typical trial were conducted by Slate *et al* (1998) who transferred more than 170 bovine microsatellites to deer and achieved a very valuable result (see Table 1).

Table 1. Results of amplifying cervid microsatellites with microsatellites primers developed in Bovine

(Slate *et al*, 1998)

Species	Primer number	Loci giving microsatellite product	Proportion giving microsatellite	Loci polymorphic	Proportion polymorphic	Mean no. of alleles (SD)
Red deer	174	129	74.1%	72	42.5%	3.4±2.9
Sika deer	171	126	73.7%	47	37.3%	1.6±1.0

The result indicates that a few loci cannot generate products in different species. The possible reason is the different sequence of flanking regions where primers are designed. Some loci are highly polymorphic in original species showed monomorphism in target species. This may cause by complex evolutionary reasons. Kuhn *et al* (1996) reported that no repeat motif was detectable after transferring some bovine micorsatellites to red deer, indicating that some non-microsatellite loci might be amplified by "mistake" in different species. Therefore, only those primers that are effective and polymorphic can be transferred to deer. To date, totally about 200 microsatellites are confirmed to be effective in cervine species.

Application of microsatellite markers in deer research

Microsatellite DNA become very powerful genetic marker because of its advantages mentioned above over other genetic markers such as RFLP, RAPD, AFLP and mtDNA. It has been used in various fields in eukaryote. For cervids, microsatellite markers are used in the following aspects:

Quantitative trait locus (QTL) selection

Most of the economic characters of deer are quantitative trait, such as antler growth, antler weight, size, shape, chemical components, body weight, utilization rate of feed, growing speed, meat quality etc. Each character is controlled by multiple genes. But the impact of these genes on the trait is not balanced. Some play key role and some are aiding factors. Because microsatellite markers distributed on chromosome in a high density, if the linkage between a microsatellite locus and a major effect gene is found, the localization of the microsatellite marker can aid to localize the gene. Mean while, the detection of the microsatellite marker can indicate the genotype or productivity of specific individual. Coulson *et al* (1998) found that 5 microsatellite loci are relative to the survival rate of red deer lambs in wintering. Some lambs possessing certain genotypes showed higher survival rate in the first and second winter than other lambs. The application of microsatellite in domestic animals has gone much further. That can be the base for relative study in deer.

As genetic marker aiding the breeding

Based on the linkage of microsatellite and QTL or corelationship of microsatellite and phenotype, identification of target character of breeding population will shorten the time and improve the efficiency of breeding. PCR-based microsatellite analysis may be performed as early as fetal period, even fertilized egg. Individuals of potentially high productivity can be raised as breeders as early as possible, and those of low productivity can be discarded as early as possible. This strategy can save expenses and achieve great progress in short period. For adult breeders, microsatellite markers help to predict the characters of offspring.

Pedigree analysis and management

Microsatellites are codominant and follow the Mendelian Laws in heredity. Therefore, paternity and maternity exclusion can be very reliable based on allelic frequency and genotype. A parentage evaluation test in North American elk indicate that overall probability of exclusion can be generated from 11 microsatellite markers of $1.52X10^{-5}$, and the probability of assigning correct parentage is 0.9951(Talbot *et al.* 1996). Another pedigree analysis of sika deer in-

volved 17 microsatellite loci. The results indicate that when mothers known and all candidate fathers are included, the mean probability of assigning correct parentage is 0.985 (Okada *et al.* 2000). In farms, or protected population, pedigree file based on microsatellites can be the base for breeding management (Glowatzki-Mullis *et al.* 1995; Bowling *et al.* 1997).

Study of population genetic diversity

Presently, we have several alternatives like mtDNA sequencing, RAPD, RFLP, AFLP, SSLP etc. to access genetic diversity. Microsatellite is a new method being used in this field. Comparatively, microsatellite has some advantages over other genetic markers. Microsatellite markers, covering whole chromosome and mutating at the rate of 10^{-4} per generation (Weber *et al.* 1993), are very informative for accessing genetic diversity. Amplified fragment length ranges from 100 to 300bp, which are very easy to amplify, despite highly degraded materials. Codominant heredity makes genotyping very simple and reliable. Polymorphism generated by variance of repeat motif can be coded in digital so that comparisons are permitted between data obtained from different experiments.

Broders *et al* (1999) focused on the genetic structure and effect of founder events on the genetic variability of moose in Canada by using microsatellite markers. The survey was based on allelic frequency of 5 microsatellite loci that are very typical methods to estimate heterozygosity of each subpopulation and divergence among them. For microsatellite markers, species- or subspecies-specific alleles can help to access the hybridization of different deer species or subspecies (Goodman *et al.* 1999; Goosen *et al.* 1997; Tate *et al.* 1998). Phylogeorgraphic relationship accessed with microsatellite data reflects the genetic distances among different hierarchical levels (Broders *et al.* 1999). The same kinds of applications in other species appear more frequently.

As genetic markers, microsatellites have some drawbacks. A variety of problems associated with the PCR process are being apparent. Non-amplification of certain alleles due to substitutions, insertions or deletions within the priming sites can lead to apparent "null" alleles which results in mistyping of heterozygotes appearing in population studies. Taq polymerase generated slippage products are routinely seen in mono-and dinucleotide repeat loci, which can sometimes make allele scoring problematic. The tendency that Taq polymerase adds an additional dATP to the end of PCR products can cause single base shifts and additional sizing problems. Each microsatellite locus is usually in just one copy per cell, poor quality and small quantity of materials, especially for non-invasively sampled materials, may generate non-specific products or allele "dropout". However, these drawbacks are common to all instead of specific problems. Proceedings and solution strategies in other species can help to solve the same problems in deer. So microsatellite is indeed a perfect genetic marker in deer research.

Future work in cervids

There is a long way to go for deer in the following aspects:

Isolate microsatellites markers specifically in cervids. Microsatellite markers are being used in many aspects, present number of microsatellite markers found in deer can not meet the demand. Large-scale isolation by molecular cloning can fulfill the shortage.

Construct genetic map and physical map of cervine microsatellites. Microsatellites are powerful tools to access the QTL and gene localization on chromosome. In this process, two problems should be solved beforehand: the linkage of microsatellite maker to the interested gene, and the location of the microsatellite marker on the chromosome. Therefore, we must have localized microsatellite markers enough to cover a major span of chromosome. Presently, genetic and physical mapping are based on hybridizing somatic cell panel with the aide of computer. Any optimization of these techniques can doubtlessly improve the process.

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